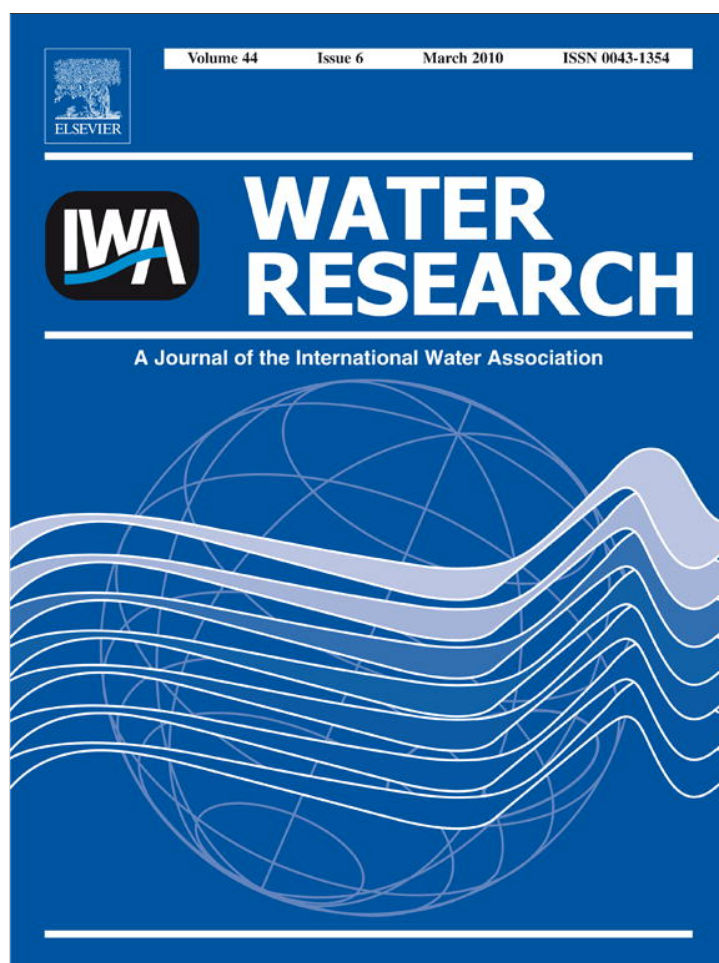


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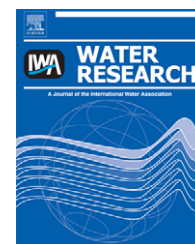


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Ecotoxicological evaluation of Mediterranean dredged sediment ports based on elutriates with oyster embryotoxicity tests after composting process

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ABSTRACT

The ecotoxicological effect of dredged sediments was estimated by embryo-larval toxicity of the oyster *Crassostrea gigas* in sediment elutriates (filtered and unfiltered). The study covers the main ports from the French Mediterranean coast. Composted sediments from a navy harbour (A), a commercial port (B) and two composite specimens (C and D) obtained after mixing various sediments were taken into consideration. Effective concentrations affecting 50% of larvae (EC50) were obtained from different elutriate concentrations (from 0 to 100%). Toxicity results obtained from filtered elutriates decreased according to the following gradient: sample A (5.68%), B (20.50%), C (37.60%) and D (47.17%). Chemical concentrations in whole sediments were in agreement with those in elutriates. Among the measured contaminants in elutriates, Cu and Zn resulted as the main contributors to toxicity. Dissolved organic carbon played an important role by exerting a protective effect against the toxicity of dissolved Cu. Toxicity results were interpreted on the basis of toxicity scores to give indication about sediment quality which provided more severe judgement than risk score based on chemical concentrations in sediments.

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1. Introduction

The maintenance dredging operations for coastal ports and waterways consist mainly in removing sediments to improve the ship traffic. In France, 50 million m³ of sediments are dredged out each year from the main maritime and commercial ports. The management of dredged sediments is a priority issue more especially in the Mediterranean Sea where sediments are historically polluted (RNO, 1998). It has become evident that chemical analysis alone is inadequate for

a full assessment of the quality of marine sediments, thus strategies for marine pollution monitoring are now integrating chemical and biological parameters. New guidelines for the disposal of dredged materials have been prepared internationally and they can be used to classify sediment samples with regard to their potential for toxicity, to identify contaminants of concern and to prioritise areas of concern based on the frequency and degree to which guidelines are exceeded (Long and MacDonald, 1998). Different authors proposed classification of toxicity by comparing biological

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responses considering sampling sites from polluted areas and groups of reference sites (Stronkhorst et al., 2003). Losso et al. (2007) proposed another approach developing toxicity scores based on a statistical method in order to classify the toxicity of elutriates deriving from sediments of an unstable environment such as the Venice lagoon where re-suspension phenomena are intense. In order to facilitate the ecological risk assessment of dredged harbour sediments and related decision making processes, the French Research Institute for Exploitation of the Sea (IFREMER) and the inter-ministerial group GEODE realised the software GEODRISK (Alzieu and Quiniou, 2001), that is based on the results of the chemical analyses (metals and PCBs) recommended in the inter-ministerial order dated 14th June 2000 and on two levels N1 and N2. The GEODRISK tool enables to distinguish between dredged sediments according to their contamination level and their potential toxicity, thus providing assistance for their management. The compliance with N1 enables the offshore-sediment dumping, conversely if N2 threshold is passed, sediment sea dumping is not allowed requiring the application of decontamination procedures. Between level N1 and level N2, a complementary investigation may turn out to be necessary according to the project considered and the excess rate over level N1 (Alzieu and Quiniou, 2001).

Recently, many works have reported the toxicity of dredged sediments as regards their disposal on land (Perrodin et al., 2006; Piou et al., 2009; Prokop et al., 2003) but few studies dealt with the ecotoxicity assessment of treated sediments. In a previous work, Libralato et al. (2008) evaluated toxicity of sediments from Venice lagoon treated via hydrocyclone and centrifuge technologies. They found that treated sediments continued generating both sub-chronic toxicity effects, which were mainly attributed to the presence of residual chemicals such as metals and PAHs.

In the present paper, the possibility of reducing sediment toxicity was investigated via mixing more and less contaminated dredged sediment batches. Sediment samples were provided in the framework of the monitoring project SED-IMARD management of dredged contaminated sediments launched in 2002 by the French public administration from the Var area in collaboration with port authorities from France and Italy (Grosdemange et al., 2008). Four sediment samples were studied: two sediments A and B dredged from the Côte d'Azur area while two others (C and D) resulted from the mixture of sediments in various proportions in order to reduce their level of contamination. This mixture procedure was considered as a pre-treatment prior to composting which consists in humidification and aeration of dredged sediments for microbial degradation.

Among the toxicity tests considered for sediment quality assessment, the oyster (*Crassostrea gigas*) embryo-larval development test was selected to investigate sediment elutriate since previous studies demonstrated that it is one of the most sensitive and rapid bioassay to check sediment samples considering sub-chronic effects (Geffard et al., 2002; Libralato et al., 2007; Quiniou et al., 2007). The use of elutriates provides information on the water-soluble constituents potentially released from the sediment to the water column. Elutriates are recommended in cases of sediment dredging activities and re-suspension phenomena in aquatic

environments for their ability to give information on potential effects towards sensitive species including gametes, embryos and larvae (Beiras, 2002; Nendza, 2002).

Sediments were dredged from different ports along the French Riviera from Marseille to the Italian boarder. Actually, that coastal area is known to be contaminated by mineral and organic compounds (Andral et al., 2004; Damiens et al., 2007), in particular the area of Toulon (French navy harbour) is generally considered as a hot spot. In this area, the French Réseau National d'Observation (RNO) (RNO, 1998) already monitored spatial and temporal trends of contaminants finding high concentrations of metal in sediments from ship Chandler sites. High TriButylTin (TBT) concentrations were also reported from the French Riviera ($2.9 \text{ mg Sn kg}^{-1}$ for Cannes) (Cassi et al., 2008).

Whole sediments were analyzed for particulate organic carbon, metals, organotins, PAHs and PCBs after dredging and composting procedures. Toxicity to oyster embryos, metals, sulphide, ammonia and dissolved organic carbon concentrations were measured on elutriates.

2. Materials and methods

2.1. Sediment dredging and sample collection

Sediments were collected from the French Mediterranean coast using a Shipek grab and laid down over a geotextile in a terrestrial disposal site. Four homogeneous sub-samples were taken into consideration: two sediments from the Côte d'Azur area (navy harbour, sample A and commercial port, sample B), and two others (samples C and D) resulted from the mixture of sediment A, B and other sediments in various proportions (Table 1). Sediments were actively composted for four months under sun light. During that time, once a week sediment mechanical turn over and humidification with freshwater took place in order to favour the microbial activity, lower sediment salt content and decrease organic pollutant concentrations. All specimens were stored at $4 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$ in the darkness before analyses for no more than three months.

Table 1 – Composition of sediment samples (expressed as percentages). C is the mixture of samples A, B and sediments from one commercial port and four pleasure ports, whereas D is composed by B and sediments from pleasure ports.

Samples	Source	Composition (%)
A	French navy harbour	100
B	Commercial port	100
C	A	20
	B	15
	One commercial port	10
	Four pleasure ports	55
D	B	25
	Three pleasure ports	75

2.2. Elutriate preparation

Elutriates were prepared according to the [AFNOR \(1998\)](#) standard protocol with Artificial Sea Water (ASW) as leaching medium as recommended by [ASTM \(2004\)](#). The 1:10 sediment: water ratio, used for evaluating the potential effects of waste material ([AFNOR, 1998](#)), was normalized to dry mass weight after 24 h at $105^{\circ}\text{C} \pm 5^{\circ}\text{C}$. Elutriates were obtained using the following steps: (1) leaching of the sediment: water mixture at 60 rpm for 24 h at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ using a Jar test (Vittadini, Padova, Italy); (2) centrifugation of the supernatant at 3000 g for 15 min (Beckman Ultracentrifuge) and filtration with $0.45\ \mu\text{m}$ sterile acetate nitrate filter. In addition, an aliquot of each elutriate sample was not filtered in order to check the influence of particulate matter in toxicity definition. All treatments were accompanied by a procedural blank preparation. Samples for chemical analyses were acidified with HNO_3 and stored at $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in the darkness until analysis.

2.3. Whole sediment physico-chemical analyses

The water content (%) was determined after gravimetric assessment drying sediment samples in three replicates at 105°C for 24 h. The percentage of fine particle ($<63\ \mu\text{m}$) was determined by laser granulometer (Mastersizer 2000, Malvern Instruments) for both raw and composted sediments. Whole sediment chemical data were obtained from SEDIMARD project ([Grosdemange et al., 2008](#)).

Organotins (TBT, DBT and MBT) analyses were carried out by the Ultra Trace Analyses Aquitaine (UT2A) Laboratory of Pau (France). After extraction in acetic acid media, organotin derivatisation was performed by adding tetraethylborate solution. After centrifugation TBT was analyzed using a quadrupole instrument and Gas Chromatography-tandem Mass Spectrometry (GC-MS-MS).

For PAH determination, sediments were submitted to microwave extraction using an acetone petroleum ether (50:50) mixture, before using high pressure liquid chromatography HPLC 1100 (Agilent) combined with variable wavelength fluorometer. PAHs that we took into consideration are those recommended by German regulation (Fluoranthene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(ghi)perylene, Indeno(1,2,3-cd)pyrene) for dredged sediments ([IMO, 2004](#)). PCBs were extracted in the same manner as PAHs and analyzed by gas chromatograph 6890 Agilent 5973 MSD equipped with mass spectrometry detection. Total concentration in PCBs is defined as the sum of seven PCB congeners (PCB 28, 52, 101, 118, 138, 153, 180) recommended by French regulation ([Alzieu and Quiniou, 2001](#)).

Particulate Organic Carbon (POC) was measured by thermal oxidation followed by infra-red detection.

In order to determine metal concentrations, 0.25 g of freeze-dried sediments were digested with a mixture of nitric acid (HNO_3 70%), oxygen peroxide (H_2O_2 30%) and fluorhydric acid (HF 48.9%) in a microwave oven. Inorganic pollutants (As, Cd, Cu, Mo, Ni, Pb, Se and Zn) were then determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

The analytical quality of chemical data was controlled with standard certified materials. Organotins and PAH concentrations were analyzed in BCR-462 and IRM-109, respectively, that were provided by the Community Bureau of Reference, Commission of European communities. For PCBs, harbour sediment reference 536, provided by I.A.E.A. (International Atomic Energy Agency, Austria), was analyzed. Results of organics were within the limits given by the supplier. For metals, the analytical procedure was calibrated against two Certified Reference Materials (CRM) from I.A.E.A. Austria and NIST-USA (IAEA-405 and SRM-2702, respectively) used in duplicates. Values obtained are shown in [Table 2](#). The recovery was satisfactory for all elements. No certified values exist for Mo in these CRMs.

2.4. Elutriate chemical analyses

The pH was measured using a pH-meter (perpHecT LogR meter, model 330, Orion, Beverly, MA, USA). The pH value was adjusted with HCl 1 M or NaOH 1 M addition when it did not fall in the toxicity test acceptability range (7.5–8.3) ([Libralato et al., 2007](#)).

The Dissolved Organic Carbon (DOC) was determined via a TOC-5050 (Shimadzu) device according to [APHA \(1998\)](#). Filtered ($0.45\ \mu\text{m}$) samples were diluted 25 times to prevent salinity interference and inorganic carbon was stripped away through air bubbling for 30 min at $20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ after acidification with H_3PO_4 . Total ammonia and sulphide concentrations were measured with a spectrophotometer (DR/2010, HACH, Loveland, CO, USA) using the salicylate ([Reardon et al., 1966](#)) and methylene blue methods (USEPA SM 4500-S2 D), respectively.

Inorganic pollutants (As, Cd, Cu, Mo, Ni Pb, Se and Zn) were measured by ICP-MS (ELAN DRC II) on filtered elutriates.

2.5. Embryotoxicity test with *C. gigas* on elutriate samples

The sub-chronic test with *C. gigas* is based on the observation of embryo-larval development abnormalities. Conditioned oyster adults were purchased from the Guernsey Sea Farm Ltd. hatchery (Guernsey, UK) and the test was carried out on the basis of [Libralato et al. \(2008\)](#). Adults spawned by thermal stimulation. A pool of eggs from at least three females (1000 mL) was fertilised by injecting 10 mL of sperm

Table 2 – Analysis of the certified reference sediments: IAEA-405 and SRM-2702.

CRM	Elements (mg/kg)	As	Cd	Cu	Ni	Pb	Se	Zn
IAEA-405	Certified values	23.6 ± 0.7	0.73 ± 0.05	47.7 ± 1.2	32.5 ± 1.4	74.8 ± 2.2	0.44 ± 0.12	279 ± 7
IAEA-405	Measured values	22.4 ± 0.7	0.74 ± 0.02	47.5 ± 3.0	32.2 ± 1.5	65 ± 1	0.45 ± 0.08	244 ± 3
SRM-2702	Certified values	45.3 ± 1.8	0.82 ± 0.1	117.7 ± 5.6	75.4 ± 1.5	133 ± 1	4.95 ± 0.46	485 ± 4
SRM-2702	Measured values	41.8 ± 0.1	0.81 ± 0.02	123 ± 1	74.5 ± 1.5	135 ± 8	4.96 ± 0.02	439 ± 7

suspension; the fertilisation was verified by microscopy. Egg density was determined by counting four sub-samples of known volume. Fertilised eggs, added to test solutions in order to obtain a density of ≈ 20 eggs mL⁻¹, were incubated for 24 h at 24 °C and then fixed with buffered formalin. One hundred larvae were counted distinguishing between normal larvae (D-shape) and abnormalities (malformed larvae and pre-larval stages). Test result acceptability was based on negative control for: a) percentage of normal D-shape larvae $\geq 80\%$ (His et al., 1999); b) Effective Concentration affecting the 50% of the considered population (EC50) respect to the reference toxicant (Cu as Cu(NO₃)₂) falling within the acceptability range (Libralato et al., 2008).

Toxicity tests were performed in triplicate using different elutriate concentrations starting from the undiluted sample (100%). Sterile polystyrene micro-plates (3 mL 24 wells) with lids (Iwaki Brand, Asahi Techno Glass Corporation, Tokyo, Japan) were used as test chambers for toxicity tests. Dilution water (for test solutions and gametes) was artificial seawater reconstituted according to ASTM (2004) at a salinity of 34.

2.6. Data analysis

Toxicity data were expressed as EC50 on the basis of percentage of abnormalities. EC50 values with 95% confidence limits were calculated by Trimmed Spearman-Kärber statistical method (ASTM, 2004). Toxic Unit at 50% (TU50) of the population exhibiting a response was determined as 100/EC50 to provide values directly correlated to the toxicity magnitude (Losso et al., 2007). The responses for each treatment (% of abnormalities) were corrected for effects in control tests by applying Abbott's formula (ASTM, 2004), obtaining the Net Percentage of Abnormalities (NPA). Statistically, treatment was performed as reported in Libralato et al. (2008).

The GEODRISK software (Alzieu and Quiniou, 2001) was considered for data elaboration to rank dredged sediment according to its level of chemical contamination (risk score).

Moreover, toxicity data were also ranked on the basis of the *C. gigas* species-specific Toxicity Score (TS) as reported in Losso et al. (2007). Toxicity thresholds were defined on the basis of the minimum significance difference (MSD) between the control and the treatment at 0.05 α -level. The methodological approach obliged the identification of two semi-scores: one based both on the percentage of effect and toxic units for low toxic samples and one based just on toxic units for more toxic specimen. The individual MSD values were divided by the respective average negative control response and ranked in ascending order to identify the 90th-percentile of the cumulative distribution of MSDs. Thus, the Toxicity Threshold (TT, TT = 100–90th-percentile of MSD) and the Toxicity Limit (TL, TL = TT * % effect in the relative control) were calculated. Finally, the TS has been developed considering five classes identified by a score (0–4) and a synthetic judgement: absent (if sample response normalized with respect to control (S) > the minimum sample response that could be regarded as significantly no different from the control (TL), score 0), low (if 50% effect < S \leq TL, score 1), medium (if 1 \leq TU50 < 2, score 2), high (if 2 \leq TU50 < 4, score 3) and very high (if TU50 \geq 4, score 4).

3. Results

3.1. Chemical data in whole sediments

Results (POC, water contents and metal concentrations) for raw and treated (composted) sediments are displayed in Table 3. Metal concentrations (As, Cd, Cu, Mo, Ni, Pb and Zn) generally decreased after composting procedure for all sediment samples except for sample B.

Raw sediments present a higher water content > 50% in A and B than in C and D $\sim 30\%$. After composting, water content decreased in all samples, particularly in A and B by 26 and 46%, respectively. Fine particle (<63 μ m) ranged as follows B >> A > C > D in both raw and treated sediments.

Table 3 – Chemical analyses in total sediments after dredging and composting procedures; POC = particulate organic carbon.

Parameters	Units	A		B		C		D	
		raw	treated	raw	treated	raw	treated	raw	treated
Sediment									
Water content	%	57.30	15.6	58.3	27.21	31.4	25.4	32.9	21.30
Fine particle (<63 μ m)	%	38.3	21.8	82.9	64.7	34.6	28.4	32.8	28.2
POC	g kg ⁻¹ d.m.	102	69	53	45	40	43	41	36
As	mg kg ⁻¹ d.m.	196	129	30	43	35	35	19	14
Cd	mg kg ⁻¹ d.m.	5.9	4.6	1.0	1.1	1.9	1.5	0.7	0.8
Cu	mg kg ⁻¹ d.m.	1459	902	206	241	380	362	243	174
Mo	mg kg ⁻¹ d.m.	16	10	20	18	15	11	19	16
Ni	mg kg ⁻¹ d.m.	42	28	33	30	27	16	29	19
Pb	mg kg ⁻¹ d.m.	1340	896	278	271	354	400	159	116
Se	mg kg ⁻¹ d.m.	<2	<2	<2	<2	<2	<2	<2	<2
Zn	mg kg ⁻¹ d.m.	3493	1932	488	611	1007	865	374	242
MBT	μ g Sn kg ⁻¹	2888	2126	186	166	548	452	672	382
DBT	μ g Sn kg ⁻¹	1924	1028	167	165	1476	935	676	562
TBT	μ g Sn kg ⁻¹	9576	6398	386	323	4271	3001	1372	941
PAHs	mg kg ⁻¹ d.m.	78.56	31.34	7.41	7.36	15.18	9.33	4.71	2.80
PCBs	mg kg ⁻¹ d.m.	0.89	0.73	0.24	0.23	0.51	0.41	0.22	0.21

Table 4 – Total and normalized chemical parameters in elutriates.

Parameter	A		B		C		D	
	LOD ^a							
pH	–	8.00	7.69		8.00		8.00	
N-NH ₄ ⁺ (mg L ⁻¹)	0.001	1.00	0.46		0.18		0.15	
S ²⁻ (mg L ⁻¹)	0.001	<0.001	<0.001		<0.001		<0.001	
		Total	Normalized values ^b	Total	Normalized values ^b	Total	Normalized values ^b	Total
DOC (mg L ⁻¹)	0.05	66.93	3.80	75.35	15.44	27.07	77.10	36.37
As (μg L ⁻¹)	0.01	34.31	1.95	26.86	5.50	11.60	28.23	13.32
Cd (μg L ⁻¹)	0.01	22.82	1.30	4.70	0.96	0.85	0.70	0.33
Cu (μg L ⁻¹)	0.31	175.64	9.97	46.53	9.53	16.67	40.72	19.21
Mo (μg L ⁻¹)	0.33	57.52	3.27	343.69	70.43	67.48	246.34	116.20
Ni (μg L ⁻¹)	0.33	25.52	1.45	16.05	3.29	6.04	12.48	5.89
Pb (μg L ⁻¹)	0.11	3.90	0.22	<0.11	–	–	<0.11	–
Se (μg L ⁻¹)	0.02	147.80	8.39	149.88	30.71	53.84	150.64	71.06
Zn (μg L ⁻¹)	1.08	475.96	27.03	56.96	11.67	5.00	15.69	7.40

a LOD—Limit of detection.

b Normalized values—normalized concentrations by the ratio 100/EC50 for each sample.

Particulate organic carbon (POC) decreased after composting in all samples except in C. In treated sediments, POC is lower in sample D than in other samples.

When considering pollutant concentrations in raw and treated sediments, sample A is the most polluted sample, except for Mo and Se. Sample B shows lower levels of Cu, Pb, Zn, organotin, PAH and PCB concentrations than sample C. Sediment D is less contaminated compared to the other ones, except for organotins.

After composting procedure, metal concentrations generally decreased in samples A, C and D whereas they increased in B. Organotin and PAH concentrations significantly decreased in samples A, C and D and to a lesser extent in sample B. PCBs are not different in raw and treated sediments.

3.2. Chemical data in elutriates

Results are presented in Table 4. Ionised ammonia concentrations decreased in the following order: A > B > C > D. Sulphide concentrations were in all cases lower than the limit of detection of the selected method. The lowest DOC is found in A and the highest in D. Elutriates A and B presented higher concentrations of inorganics (Cu, Zn and Cd) than mixed sediments (Sample C and D). Mo was found in all samples, but in high concentration in sample B, whereas As and Se were present at the same level in all samples.

3.3. Toxicity tests

Negative controls were carried out considering both filtered (0.45 μm) and unfiltered Artificial Sea Water (ASW) (ASTM, 2004). The negative controls from filtered ASW presented 15% ± 2% effect (as the sum of abnormalities and not developed larvae) (n = 3), while the unfiltered ones 9% ± 2% effect (as the sum of abnormalities and not developed larvae) (n = 3). They were both acceptable, i.e. <20% effect (ASTM, 2004). The positive control presented an EC50 value of 10.98 μg Cu L⁻¹ (10.11 μg Cu L⁻¹–11.93 μg Cu L⁻¹) (n = 3), that is in accordance with Libralato et al. (2008).

The ecotoxicological assessment of filtered elutriates expressed as EC50 values (% elutriate sample) evidenced the

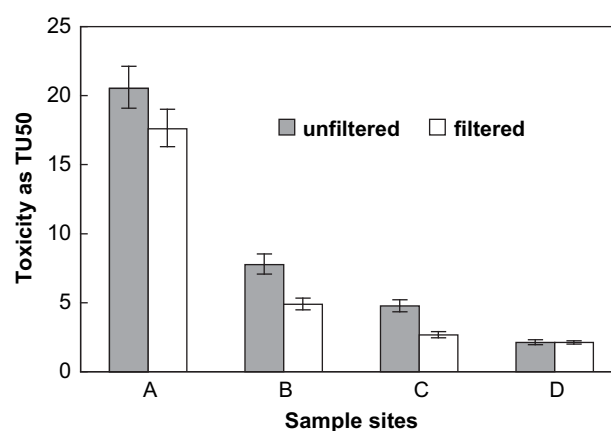


Fig. 1 – Toxicity trend as TU50 (toxicity unit) based on EC50 (TU50 = 100/EC50) established from the percentage of abnormal development of *Crassostrea gigas* embryos for samples A, B, C, D (x axis).

following gradient: 5.68 (5.26–6.13) for sample A, 20.50 (18.79–22.36) for sample B, 37.60 (34.52–40.96) for sample C and 47.17 (44.44–50.08) for sample D. Moreover, the unfiltered elutriates presented the same EC50 gradient ($A < B < C < D$). It has been evidenced that the filtration process removed part of the toxicity that could be attributed to the suspended particulate matter, except for D that did not significantly varied. In particular, the toxicity of A was reduced by a factor 1.2, that of B of a factor 1.6, while that of C of a factor 1.8. Unfiltered and filtered toxicity data as TU50 are displayed in Fig. 1.

4. Discussion

Results of chemical pollutants in whole sediments confirmed that sample A is significantly contaminated by TBT and metals. Indeed, it is located in a ship chandler zone where antifouling paints releasing Cu and organotins are used (Cassi et al., 2008). In addition, intensive maritime activities occurs releasing PAHs into the environment. Conversely, sample B presented much lower pollutant concentrations especially TBT, while Cu content was similar to that of C and D. The mixture of sediments was performed by port authorities in order to decrease the contamination levels of samples A and B. Sample C reflected the dilution of sample A, sample B and the contribution of a commercial port, whereas sample D originated from the dilution of B and other pleasure ports. Thus the composition of the mixture might explain the difference in chemical contents of C and D.

The composting procedure decreased significantly organotin and PAH concentrations (Table 3) in sediment samples (A, C and D) that may be explained by the high microbial activity induced by the moisture changing treatments (Saeki et al., 2007; Vermeulen et al., 2003). PCBs which are anthropogenic contaminants are not degraded by endogenous bacterial activity. Metal (mainly Cu and Zn) concentrations increased in sample B, where the highest quantity of fine particles is found. In sediments, metals are mainly present in the clay-silt particles with grain size lower than 63 μm , due to the high specific surface properties of this fraction (Chen et al., 2004; De Gregori et al., 1996; Ujevic et al., 2000). The low effect of composting procedure in sample B may be due to the finer

particle size which limits sediment oxygenation and therefore aerobic biodegradation (Vermeulen et al., 2003).

To evaluate the sediment contamination and potential ecotoxicological effects associated with the observed concentrations of contaminants GEODE sediment quality guidelines have been used to compute risk score with GEODRISK software (Alzieu and Quiniou, 2001) (Table 5). Risk score is higher for A and the same for B and C, whereas it is lower for D. In particular, risk score for A is higher than two. In this case, immersion of dredged sediments cannot be authorised, due to the fact that sediments are considered as wastes and must be kept out of water, and consequently an impact study is required to evaluate the potential toxicity of waste elutriates. For other samples (B, C and D), the contaminant risk score is between 1.4 and 2.1, therefore a local impact study must be directly carried out in order to check if sediment immersion may represent the less harmful solution for the environment.

In the present study, an integrated approach was achieved combining elutriate chemistry and toxicity tests with oyster larvae.

Ammonia and sulphide were measured since these compounds, derived from the natural processes of decomposition, can accumulate in sediment causing toxic effect (Gagné et al., 1999). In the present work, ammonia and sulphide had no role as confounding factors. Indeed, ammonia concentrations were lower than the No Observed Effect Concentration (NOEC) of 4.7 mg L^{-1} (ASTM, 2004) for ionised ammonia. All sulphide concentrations were under the NOEC of 0.1 mg L^{-1} (ASTM, 2004), which is considered as the sensitivity threshold limit value of bivalve embryotoxicity test towards sulphide.

Chemical concentrations in total elutriates (Table 4) are correlated with those found in total sediments (Table 3). DOC concentrations are particularly high in the considered elutriate samples compared to those reported by Geffard et al. (2002). These authors observed higher toxicity levels from freeze-dried sediments in comparison with fresh ones measured with *C. gigas* embryotoxicity test. They hypothesized that the increase of DOC concentration in elutriates from rewetting freeze-dried sediments may be related to the fact that freeze-dried sediments are better dispersed (i.e. disaggregated) than fresh ones and may increase the bioavailability of contaminants. In the present paper sediments were submitted to several cycles of dry and rewetting processes during storage

Table 5 – Classification of toxicity in the studied samples according to Losso et al. (2007) and risk scores following GEODRISK recommendations (Alzieu and Quiniou, 2001).

		TU50	Toxicity ^a	Risk Score ^b
Unfiltered	A	20.53 (19.08–22.12)	Very high (4)	2.7
	B	7.76 (7.06–8.53)	Very high (4)	2.1
	C	4.75 (5.21–4.33)	Very high (4)	2.1
	D	2.12 (5.21–4.33)	High (3)	1.4
Filtered	A	17.61 (16.31–19.01)	Very high (4)	2.7
	B	4.88 (4.47–5.32)	Very high (4)	2.1
	C	2.66 (2.44–2.90)	High (3)	2.1
	D	2.12 (2.00–2.25)	High (3)	1.4

^a Losso et al. (2007).

^b Alzieu and Quiniou (2001).

and composting, similarly to Geffard et al. (2002), that might have enhanced their desegregation thus explaining the high DOC levels and contaminant bioavailability.

In order to explain the contribution of contaminants to toxicity, the concentrations of each pollutant as well as DOC were normalized at the dilution concentration of filtered elutriate samples generating EC50s as displayed in Table 4. DOC is low in A, and increases from B to D. Adjusted concentrations increase from A to D for As, Cu, Mo, Ni and Se, while sample A presents the highest levels of Cd, Co and Zn.

The comparison between metal concentrations in elutriate samples at EC50 values (Table 4) and their relative EC50s as pure substances with *C. gigas* embryotoxicity test from literature showed that Cu could be directly involved in the potential toxicity of all samples. Indeed, Cu EC50 values in elutriates are higher than the EC50s reported in the literature ranging from 5 to 23 $\mu\text{g L}^{-1}$ (Damians et al., 2006; His et al., 1997; PAN, 2007). Arsenic, Ni and Zn did not significantly contribute to elutriate toxicity. The EC50 values for *C. gigas* ranged between 326 and 920 $\mu\text{g L}^{-1}$ for As (Martin et al., 1981; PAN, 2007), for Ni the EC50 is around 39–250 $\mu\text{g L}^{-1}$ (Martin et al., 1981; PAN, 2007; Robert and His, 1985) and 119–250 $\mu\text{g L}^{-1}$ for Zn (Brereton et al., 1973; Martin et al., 1981; PAN, 2007). Moreover, Mo and Se oxyanions did not seem to participate to the toxicity of elutriates.

Samples containing the lowest DOC present the greatest toxicity and therefore significant bioavailability of metal contaminants. Nadella et al. (2009) demonstrated that experimental addition of DOC in natural seawater reduced Cu toxicity. For example, the EC50 of the mussel *Mytilus trossulus* embryos developing in seawater with 20 mg DOC L^{-1} was 39.00 $\mu\text{g Cu L}^{-1}$ (35.20–47.20) corresponding in a 4-fold increase in Cu EC50, highlighting the protective effect of DOC against the toxicity of dissolved Cu.

Toxicity of elutriates cannot be explained only by single metal EC50 values and the content of DOC. Indeed, a more complex response such as synergism effect must be considered as a potential contribution to the final toxicity of samples. Fernandez and Beiras (2001) have observed additive or very slightly synergistic effects for combinations of Hg with Cu, Pb and Cd. Additive effects have been observed in bivalves for mixtures of Cu–Ag and Mn–Mo (Morgan et al., 1986). MacInnes (1981) found additive effects for Hg–Cu and Hg–Zn combinations, and a synergistic interaction for a Cu–Zn mixture. Libralato (2007) reported the toxicity of Cd, Cu, Ni and Zn as single metal and also as a five-metal mixture for the oyster larvae *C. gigas* in artificial seawater. This author showed that EC50s of the five-metal mixtures was situated at concentrations of about 10% of the EC50 of the individual metals in seawater and at about 33% and 55% when 5 and 10 mg L^{-1} of fulvic acid were added. In elutriates, Cu and Zn are the main toxic metals to oyster larvae due to their bioavailability and to the synergistic effect between themselves and other contaminants. However, organic contaminants (TBT, PAHs and PCBs found in whole sediments) that were not measured in elutriates could have toxic effects on *C. gigas* larvae.

Among the regulatory requirements proposed for discriminating sediment toxicity, the classification elaborated by Losso et al. (2007) was discussed. Filtered elutriates from samples A and B were very highly toxic, whereas samples C and D were highly toxic as displayed in Table 4. Moreover, for

unfiltered elutriate toxicity, besides A and B, C could also be classified as very highly toxic, while only D was highly toxic. Geffard (2001) reported that sediment particles had no mechanical adverse effects on embryos of *C. gigas*, but in this specific case, elutriate suspended solids might have transported additional pollutants adsorbed onto residual sediment particles. The results of TU50s show that the immersion of all sediments samples might represent a significant ecological hazard particularly in the case of transitional environments with high re-suspension phenomena.

5. Conclusions

The aim of this investigation was to evaluate in terms of toxicity and organic and inorganic contaminations the effectiveness of mixtures of highly polluted sediments (A and B) with less polluted sediments (C) and (D) resulting from the dilution of A and B with less contaminated sediments from pleasure ports, after composting treatment. The main implications of this work are the following:

- The toxicity of elutriates evidenced that mixed specimens were less toxic than unmixed ones: sample A (French navy port, unmixed) > sample B (commercial port, unmixed) > mixed sediment samples (C and D), meaning that the mixture of highly polluted with less polluted sediments is effective to decrease the global toxicity;
- The toxicity levels may be attributed to the bioavailability of Cu and Zn and the synergistic action of metal mixture, meaning that sediment toxicity cannot be predicted using metal concentrations in whole sediments;
- Composted sediments need further treatment for metal stabilization prior to beneficial re-use or long-term storage. The additional treatments should focus mainly on more bioavailable and toxic metals such as Cu and Zn.

The relation between sediment quality and ecological risk assessment is complex; requiring assessment methods based on bioavailable contaminant fractions and bioassays rather than on results obtained from traditional total contaminant concentrations.

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